

Application No.: 09/121,239
Filed: July 23, 1998
Art Unit: 1635

AFTER FINAL AMENDMENT
Docket No. GP091-02.UT
Confirmation No. 3098

a complementary splice junction site, and

a first probe binding site located in a *bcr*-derived sequence and not overlapping

the complementary splice junction site;

G¹
d) hybridizing the second nucleic acid strands with a *bcr*-specific oligonucleotide probe under hybridization conditions in which the probe hybridizes to the first probe binding site, thereby forming a probe:target hybrid; and

e) detecting the probe:target hybrid as an indication of the presence of the fusion nucleic acid in the sample.

N.E.
2. (Reiterated) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is an mRNA, the first primer is a promoter-primer, the polymerase activity comprises an RNA polymerase activity, and the oligonucleotide probe is of the same sense as the mRNA and binds to the first probe binding site.

G²
3. (Amended) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is a mRNA, wherein the second nucleic acid strands are complementary RNA, wherein the amplifying step includes contacting the second nucleic acid strand with a second primer or promoter-primer which hybridizes to a *bcr*-specific second primer binding site that hybridizes to SEQ ID NO:5, and wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity and an RNA-directed DNA polymerase activity.

4. (Canceled)

G³
5. (Amended) The method of Claim 1, wherein the *bcr*-specific oligonucleotide probe has a sequence of SEQ ID NO:9 or SEQ ID NO:27.

Application No.: 09/121,239
Filed: July 23, 1998
Art Unit: 1635

AFTER FINAL AMENDMENT
Docket No. GP091-02.UT
Confirmation No. 3098

6. (Reiterated) The method of Claim 1, wherein step a) includes preparing RNA from the sample containing the fusion nucleic acid by:

contacting a biological sample comprising the fusion nucleic acid with a solution consisting essentially of:

NIK

- a buffer,
- about 150 mM to about 1 M of a soluble salt,
- about 0.5% to about 1.5% (v/v) of a non-ionic detergent, and
- a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms, directly or indirectly, a stable hybridization complex with an RNA under conditions permitting the formation of the stable hybridization complex; and
- separating the hybridization complex joined to the solid support from unhybridized sample components without extracting the RNA using reagents such as phenol or chloroform.

7. (Reiterated) The method of Claim 6, wherein the fusion nucleic acid is mRNA.

8. (Reiterated) The method of Claim 7, wherein the nucleotide base sequence of the immobilized oligonucleotide comprises a poly-T sequence.

G⁴

9. (Amended Five Times) A method of detecting a fusion mRNA transcript produced as a result of a chromosomal translocation comprising the steps of:

- a) providing a sample containing a fusion mRNA transcript comprising a *bcr-abl* splice junction;
- b) contacting under isothermal nucleic acid amplification conditions:
the fusion mRNA transcript,

Application No.: 09/121,239

Filed: July 23, 1998

Art Unit: 1635

AFTER FINAL AMENDMENT

Docket No. GP091-02.UT

Confirmation No. 3098

a first primer which hybridizes to a sequence of SEQ ID NO:22, and
at least one enzyme having nucleic acid polymerase activity;

c) amplifying the fusion mRNA transcript in a single nucleic acid amplification reaction that uses the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the fusion mRNA transcript containing the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site derived from a *bcr* sequence and not overlapping the complementary splice junction site, and

a second probe binding site derived from an *abl* sequence and not overlapping the complementary splice junction site, wherein the second probe binding site overlaps or is located 3' to sequence complementary to SEQ ID NO:22;

d) hybridizing the second nucleic acid strands with an oligonucleotide probe which hybridizes to the second nucleic acid strands at either the first probe binding site or the second probe binding site but does not hybridize to the fusion mRNA transcript, thereby forming a hybridization complex of the first probe or the second probe and the second nucleic acid strand; and

e) detecting the hybridization complex as an indication of the presence of the fusion transcript in the sample.

10. (Amended) The method of Claim 9, wherein the amplifying step uses only a first primer that is a promoter primer of SEQ ID NO:1 and the enzyme has an RNA polymerase activity, and wherein the hybridizing step uses an oligonucleotide probe which hybridizes to the second nucleic acid at the first probe binding site.

11. (Canceled)

Application No.: 09/121,239
Filed: July 23, 1998
Art Unit: 1635

AFTER FINAL AMENDMENT
Docket No. GP091-02.UT
Confirmation No. 3098

N/E
12. (Reiterated) The method of Claim 9, wherein the first probe binding site is derived from a different chromosome than the chromosome from which the second probe binding site is derived, and the fusion mRNA transcript detected results from a translocation involving different chromosomes.

13. (Canceled)

G/E
14. (Amended) The method of Claim 9, wherein the fusion mRNA transcript results from a human t(9;22) translocation.

15. (Reiterated) One or more oligonucleotides suitable for use in the method of Claim 14, have a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:23, SEQ ID NO:26 and SEQ ID NO:27.

N/E
16. (Reiterated) The method of Claim 9, wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity, and an RNA-directed DNA polymerase activity, and further uses a second primer or promoter primer which hybridizes under amplification conditions to a nucleotide sequence of a complementary RNA produced during the amplifying step.

17. (Reiterated) The method of Claim 16, wherein the RNA-directed DNA polymerase activity and DNA-directed DNA polymerase activity are supplied by a reverse transcriptase.

G
18. (Amended) The method of Claim 9, wherein the amplifying step also amplifies an internal control normal *abl* transcript in the sample by using the first primer and then hybridizing an *abl*-specific second oligonucleotide probe which hybridizes to the complement of the internal control transcript but does not

G¹

Application No.: 09/121,239
Filed: July 23, 1998
Art Unit: 1635

AFTER FINAL AMENDMENT
Docket No. GP091-02.UT
Confirmation No. 3098

hybridize to the complement of the fusion mRNA transcript thereby forming in internal control hybridization complex, and wherein the detecting step also detects the presence of the internal control hybridization complex in the sample, thereby providing an internal standard.

19. (Reiterated) A method of preparing a sample containing RNA suitable for amplification, consisting essentially of the steps of:

- U.K.
- a) providing a biological sample comprising unpurified RNA;
 - b) mixing the biological sample with a solution consisting essentially of:
 - a buffer at a pH of about 6.5 to about 8.5,
 - about 150 mM to about 1M of a soluble salt, and
 - about 0.5% to about 1.5% (v/v) of a non-ionic detergent, to produce a solution containing released RNA;

- e) mixing the solution containing released RNA with a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions;
- d) separating the hybridization complex joined to the solid support from unhybridized sample components; and
- e) then washing the hybridization complex joined to the solid support with a solution having sufficient salt concentration to maintain the hybridization complex, thereby not requiring extraction using reagents such as phenol or chloroform to prepare RNA.

20. (Reiterated) The method of Claim 19, wherein the biological sample is uncoagulated blood, plasma or bone marrow.

G²

21. (Amended) A method of detecting a fusion mRNA transcript produced as a result of a human bcr-

Application No.: 09/121,239
Filed: July 23, 1998
Art Unit: 1635

AFTER FINAL AMENDMENT
Docket No. GP091-02.UT
Confirmation No. 3098

abl/translocation comprising the steps of:

- 18
- a) providing a sample containing a human fusion mRNA transcript comprising a *bcr-abl* splice junction;
- b) contacting under isothermal nucleic acid amplification conditions:
- the fusion mRNA transcript,
 - an *abl*-specific first primer that binds to a primer binding site of SEQ ID NO:22, which is located in an *abl*-specific sequence flanking the *bcr-abl* splice junction site,
 - a *bcr*-specific second primer having a sequence of SEQ ID NO:5, which hybridizes to a *bcr*-specific sequence flanking the *bcr-abl* splice junction site,
 - at least one enzyme having an RNA-directed DNA polymerase activity, and
 - at least one enzyme having an DNA-directed RNA polymerase activity;
- c) amplifying the fusion mRNA transcript in a single nucleic acid amplification reaction that uses
- the *abl*-specific first primer,
 - the *bcr*-specific second primer, and
 - the DNA-dependent RNA polymerase activity to produce amplified RNA that is complementary to the fusion mRNA transcript comprising the *bcr-abl* splice junction;
- d) hybridizing the amplified RNA with a *bcr*-specific oligonucleotide probe which hybridizes to a probe binding site derived from a *bcr* region flanking the *bcr-abl* splice junction, thereby forming a hybridization complex; and
- e) detecting the hybridization complex as an indication of the presence of the fusion mRNA transcript in the sample.

22. (Amended) The method of claim 21, further comprising

in the contacting step, contacting an *abl*-specific third primer having a sequence of SEQ ID NO:13 that hybridizes to the complement of a normal *abl* mRNA transcript,

Application No.: 09/121,239
Filed: July 23, 1998
Art Unit: 1635

AFTER FINAL AMENDMENT
Docket No. GP091-02.UT
Confirmation No. 3098

in the amplifying step, amplifying a normal *abl* sequence present in the normal *abl* mRNA by using the *abl*-specific first primer and the *abl*-specific third primer,

in the hybridizing step, hybridizing an oligonucleotide probe that hybridizes to a probe binding site derived from an *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript, and

in the detecting step, detecting a hybridization complex made up of the probe hybridized to an *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript, thereby providing an internal control based on amplifying and detecting normal *abl* sequence.

23. (Amended) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5, and the *bcr*-specific oligonucleotide probe is of SEQ ID NO:9 or SEQ ID NO:27 or is a mixture of SEQ ID NO:9 and SEQ ID NO:27.

24. (Reiterated) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5 or its RNA equivalent, and the probe is of SEQ ID NO:9 or its RNA equivalent.

25. (Reiterated) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5, and the probe is of SEQ ID NO:27.

26. (Reiterated) The method of claim 22, wherein the third primer is of SEQ ID NO:13 or its RNA equivalent, and the oligonucleotide probe that hybridizes to the *abl* sequence is of SEQ ID NO:16 or its RNA equivalent or SEQ ID NO:26.

REMARKS

Claims 1-26 are pending and stand rejected. The specification has been amended at page 13 and page 48. Claims 1, 3, 5, 9, 10, 14, 18, and 21-23 have been amended, and claims 4, 11, and 13 have been canceled. No new matter has been added by these amendments. Support for the